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on

RESEARCH ON DEEP SUBMERGENCE DIVING PHYSIOLOGY AND DECOMPRESSION TECHNOLOGY UTILIZING SWINE: EVALUATION OF SWINE AS A HYPERBARIC ANALOG TO MAN AND DETECTION OF EMBOLI BY USE OF THE ULTRASONIC DOPPLER FLOWMETER:

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Murlin F. Gillis, D.V.M.

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13. ABSTRACT

Sixteen miniature swine of similar size performed 83 dry chamber air dives, using no-stage profiles with linear ascent and descent rates of 60 ft/min, depths of 60 to 180 ft, and bottom times up to 120 min. Surface intervals always exceed 48 hr. Signs considered diagnostic of bends were lameness, persisting for 15 minutes or more and relieved by recompression, and/or acute, progressive respiratory distress, and/or central neurologic signs, e.g. paralysis. Bends was not observed at 60 ft with bottom times up to 120 min; a 60% incidence was recorded for 140 ft/60 min and a 16% incidence for 180 ft/20 min. Individual variation and apparent dependence on prior diving experience have produced erratic results, and statistically significant incidence figures are being sought through continued experimentation.

Doppler flowmeter studies have shown that individual glass microballoons of 80-150 micra diameter are easily detectable in the thoracic caudal vena cava, and that 40 micra balloons, while difficult to detect individually, are easily detected when injected in large quantities. Severe caval air embolism has been recorded following 180 ft/15 min excursions without bends signs, confirming that significant gas embolism can exist in the absence of such signs and showing the value of the Doppler flow meter for embolism detection. Evaluation of the Doppler flowmeter as an in vivo bubble sizer continues, but results to date are discouraging. Numerous factors, presently uncontrollable, will probably render the instrument of little value for sizing.

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OBJECTIVES

The need for an animal analog to man for hyperbaric research is well recognized. The miniature swine[1] has attracted attention in this regard because of its similarities to man in size and cardiovascular physiology and because of its relatively high fat/lean ratio. If the similarities between this species and man extend to their response to hyperbaric excursions, the miniature swine may qualify as a valuable animal model for basic research in hyperbaric medicine or even as a stand-in for man during the early stages of development of decompression procedures for man. One stated objective of our research program is to attempt to define the no-decompression limits for this species through dry chamber air dives, using as a guide the no-decompression limits for man as published in the U. S. Navy Diving Manual for 1963. Simulated depths of 60, 140 and 190 feet were scheduled for investigation this first fiscal year.

In 1967 it was discovered that the ultrasonic Doppler blood flowmeter[2], known for some time to be exquisitely sensitive to the presence of bubbles in the circulating fluid, was capable of detecting gas emboli in animals undergoing decompression. Publications on this work appeared later in the literature[3,4,5,6,7]. Immediately questions arose whether those asymptomatic gas emboli, if they exist, can be detected prior to the onset of bends. Besides its obvious applications to animal research on the kinetics of bubble formation, the instrument may be useful for monitoring decompression on an individual basis or for objectively assessing the embolic status of human subjects suffering decompression sickness.

Questions arose as to the threshold size of embolus detectible with this instrument, and whether some characteristic of the signal produced by an embolus might give a measure of its physical dimensions. The second broad objective of our research program is to continue our earlier work on the use of the Doppler flowmeter in these regards.

RESULTS

ANIMAL STUDIES

Sixteen miniature swine were used to perform a total of 83 pig-dives. Twelve were males (six castrated), four were females, and all were of the Hanford Miniature Swine or HMS-Labco Cross strains. Fourteen weighed between 105 and 128 pounds, the others weighing 92 and 154 pounds; no correlation between bends susceptibility and weight was evident. Seventy-five of the 83 pig-dives were performed by normal, unoperated animals; eight pig-dives were performed using animals on whose thoracic caudal venae cavae had been implanted Doppler flowmeter transducers. Depths simulated by dry chamber air dives included 60, 140 and 180 feet, the latter substituted for 190 feet on the recommendations of Surgeon Commander J. S. P. Rawlins, Royal Navy, who cited a 12% incidence of bends in R.N. divers performing 180 feet/20 minutes no-decompression excursions. All dives were of the no-decompression type, with linear ascent and descent at 60 feet/minute and bottom times up to 120 minutes. Surface intervals always exceeded 48 hours. Animals were coaxed to enter and leave the chamber without rough handling, and post-dive observations were made as the animals moved freely in an outside walkway or in their pens. Close observation of behavior continued for two hours postdive, with intermittent checks made for the following 24 hours. Husbandry, including feed composition and amount, was virtually identical for all animals.

Symptomatology

Since animals cannot relate symptoms, assessment of their physical condition is made on the basis of signs presented. A number of different signs were commonly observed (Table I), and it became necessary to define what sign or set of signs constituted decompression sickness. As we gained experience in the response of this species to hyperbaric excursions, the following were defined to be diagnostic signs of decompression sickness: (1) Lameness, muscular fasciculation or weakness in any limb, persisting for 15 minutes or longer and relieved by recompression, and/or (2) Acute, progressive respiratory distress, with or without cyanosis, and/or (3) Central neurologic signs, e.g., dysmetria, paralysis, convulsions, unconsciousness. Temporary ataxia, incoordination, loss of balance, head shaking and tilting, and even vomiting were interpreted as being of vestibular origin, a result of pressure changes in the middle ear, and were not considered signs of bends.

It must be stressed that milder symptoms, such as those reported by human subjects, may have been experienced by these animals. For example, pruritus may or may not indicate "skin bends". Drooping ears, lethargy and salivation probably indicate nausea. Certain characteristics in countenance or demeanor often gave us the impression that the animal was suffering from confusion, mild vertigo, or deep pain somewhere other than in the limbs. Total deafness has been confirmed in some cases, but subtler abnormalities such as partial deafness and

Table I. Summary of Clinical Signs Observed in 83 Pig-Dives.

Depth (Feet)	Bottom Time (Minutes)	Number of Dives Performed	Signs (Number) and Remarks
60	60	16	Ascent head shaking (13), pruritus (6), vomiting and lethargy (1), ataxia (1).
	90	2	No signs.
	120	5	Ascent ataxia (1), vomiting (2).
140	15	15	Ascent head shaking (13), ascent ataxia (5), descent head shaking (2), pruritus (1), vomiting, lethargy and ataxia following surfacing (1).
	30	6	Ascent head shaking (5), ascent ataxia (4), pruritus (1), persistent vomiting (1), total hearing loss (1), temporary inability to rise following surfacing (2).
	40	2	Ascent head shaking (2), ascent ataxia (2), descent ataxia (1), muscular tremors after surfacing (1), vomiting and ataxia (1). None treated.
	60	5	Bottom ataxia (2), ascent head shaking (2), vomiting (2), pruritus (2), rear leg weakness and lameness (1). Also two acute deaths preceded by ataxia, dysmetria, dyspnea, convulsions, hematemesis, paralysis and unconsciousness. Three bends, one successfully treated by recompression.
180	0	1	Bounce dive on animal habitually showing ataxia and vomiting. No signs observed.
	10	5	Ascent head shaking (4), vomiting (1).
	15	2	Ascent ataxia (1), vomiting (1), descent ataxia (1), bottom ataxia (1), persistent incoordination (1). Both Doppler preparations, many caval emboli. No treatment required.

Table I. Summary of Clinical Signs Observed in 83 Pig-Dives, Cont.

Depth (Feet)	Bottom Time (Minutes)	Number of Dives Performed	Signs (Number) and Remarks
180	20	19	Ascent head shaking (8), ascent ataxia (4), pruritus (3), ascent unconsciousness, temporary (1), persistent ataxia, vomiting and lethargy (2), progressive lameness (2), temporary lameness (1), one Doppler preparation (anesthetized) revealed massive gas embolism of thoracic caudal vena cava. Three cases of bends, all treated successfully.
	30	5	Ascent head shaking (1), temporary rear leg lameness, not treated (1), frothy ascitic fluid found at necropsy (1), progressive dyspnea (4), restlessness (1), cyanosis (4), progressive signs of lameness, ataxia, incoordination, weakness, paralysis and death in spite of recompression (3), vomiting (1). Four bends cases, three died in spite of recompression and one successfully treated.

tinnitus can be diagnosed in animals only with great difficulty if at all. Mild visual defects are equally difficult to detect. Thus our conclusions are based on clinically observable signs only, though experienced personnel working with swine as test animals for decompression procedures will undoubtedly atune themselves to more subjective indicators of decompression sickness in this species.

Results

Omitting the 180-foot bounce dive, the results of 82 pig-dives may be summarized as in Table II, where bends is defined as above.

Table II. Bends Incidence in Miniature Swine

Depth (feet)	Bottom Time (minutes)	Number of Pig-Dives	Number of Bends Cases	%
60	60	16	0	0
	90	2	0	0
	120	5	0	0
140	15	15	0	0
	30	6	0	0
	40	2	0	0
	60	5	3	60
180	10	5	0	0
	15	2	0	0
	20	19	3	16
	30	5	4	80

Discussion

Unfortunately the picture is not as clear as Table II might imply. Marked individual variations are seen between animals, so the number of pig-dives are inadequate for computing statistically significant incidence figures. Two pig-dives at 140 feet/40 minutes without signs of bends, for example, is

of little significance. The most striking example of individual variation was seen when two littermates of equal size performed a 140 feet/60 minutes excursion together in the chamber. Forty minutes after reaching surface one exhibited hematemesis and dysmetria, rapidly progressing to collapse and clonic convulsions. The littermate showed no signs whatsoever. Both were returned to the chamber where, despite recompression to 200 feet, the first animal died. At no time did the littermate seem disturbed.

It is strongly suspected, as well, that experienced animals (those having experienced a half dozen or more recent dives) are more resistant to bends than are neophytes. For example, the first fifteen 180 feet/20 minute profiles were performed by five animals, each of whom had recently completed 3 dives each at 60 and 140 feet (without bends). Although no signs of bends were observed following these 180-foot excursions, three of the subsequent four trials of this profile -- using neophyte pigs -- produced bends. If this phenomenon can be unequivocally confirmed, an animal model is available for the study of acquired bends resistance. In the meantime, however, such a profound dependence on prior diving experience confounds progress in defining the no-decompression limits for this species.

Pigs exhibit signs of ear pain and vestibular disturbance (head shaking, temporary ataxia or loss of balance) almost exclusively during ascent. Such signs are seen to some extent on virtually all ascents,

whereas similar signs appeared during only 4 of the 83 descents and in only 2 of the 16 animals. Since man typically has greater difficulties during descent, it appears there is a difference in pharyngeal/eustachian/middle ear architecture between the species. We have performed no comparative anatomical study.

All animals which died as a result of their hyperbaric excursion were immediately examined at necropsy. All had widespread and massive gas embolism of both arterial and venous systems, usually to the extent that the blood was foamy in appearance. No grossly visible vessels were free of embolization. One animal, ascitic as a result of caval closure under an implanted Doppler flow transducer, was sacrificed immediately after displaying transient postdive lameness in one rear leg (180 feet/30 minutes). No intravascular emboli were grossly visible, but the accumulated ascitic fluid was covered with foam.

Movies (Super-8 mm) of miniature swine exhibiting various signs of bends have been made and are constantly being improved in scope and quality. They are available for sponsor use at any time.

DOPPLER FLOWMETER STUDIES

In Vivo Threshold for Embolus Detection

Graded microbeads and microballoons of glass were injected via the femoral vein of an anesthetized miniature swine on whose thoracic caudal vena cava had been implanted a Doppler flowmeter transducer. The microbeads and microballoons were suspended in physiological saline and injected at rates which, except for the smallest group (37-43 micra diameter), produced individual Doppler "chirps". Practical procedural difficulties were encountered (electrostatic clumping of the particles, catheter blockage, retention of particles in the catheter or veins by adhesion to the wall, presence of microscopic gas bubbles in the carrier fluid) but were solved for the most part. Frequent injections of saline alone were made to produce "control signals" (verifying that sounds heard were actually due to particles) and to wash away adherent particles. All batches of graded beads/balloons were characterized by measurement of large numbers using a microscope with a reticule eyepiece. The results were as follows:

- Solid glass microbeads do not produce a Doppler signal as large as do hollow glass microballoons of the same size.
- Under physiologic conditions of caval blood flow, individual glass microballoons of 80-150 micra diameter are very easily detectible in the caudal vena cava using a 13 mm I.D. perivascular Doppler flowmeter, producing large, obvious, individual "chirps" on the blood flow audio background.

- Microballoons of diameter in the vicinity of 40 micra are quite difficult to detect individually in the presence of blood flow. Injected in concentrated batches, however, their passage through the transducer field is clearly signalled by an increase in flow volume and a "ragged" quality to the audio.

It is noted that these results refer to the use of a perivascular transducer on the caudal vena cava. Presumably the threshold size would be smaller if smaller vessels were used, since an embolus of given size would occupy a larger proportion of the blood flow through the transducer field. It is also conceivable that through design modifications, such as changes in crystal orientation or frequency, the sensitivity of the Doppler flowmeter to embolus passage may be substantially increased. Finally, it must be admitted that the above threshold determinations were made on glass microballoons, not gas bubbles. Though it is believed that their ultrasound scattering properties are similar (apart from resonance), this has not been confirmed.

In Vivo Gas Embolism and Bends

Perivascular Doppler blood flowmeter transducers were implanted on the thoracic caudal vena cava of five miniature swine. Routine right thoracotomy procedures were followed, the transducer coupled to the vessel with sterile agar (and, ultimately, pleural fluids), and the leads delivered percutaneously at the dorsal midline near the scapulae. Although 13 mm I.D. transducers seemed to fit well, early stenosis and

closure of the vessel occurred in most animals; we have not experienced this trouble in the past, and are still seeking the reasons for acute failure. Perhaps larger (16 mm I.D.) transducers will have to be used, though lack of good fluid coupling in the immediate postoperative period precludes early flow detection with such oversized transducers.

An anesthetized miniswine so prepared was moved directly from the operating table to the hyperbaric chamber and a telemetering flowmeter pack attached to the transducer leads. Caval flow was monitored aurally and recorded on magnetic tape via a remote radio receiver connected to a receiving antenna inside the chamber. The animal was subjected to a 180 feet/20 minutes profile (Rawlins' dive), and severe caval gas embolism was detected. In fact, periodic loss of flow signal suggested "foam flow". The animal was sacrificed and embolism confirmed at necropsy. A second miniswine, fully recovered from transducer implant surgery, was fitted with a harness[8] holding the flowmeter telemeter unit and placed in the chamber, unanesthetized. Following a 180 feet/15 minutes excursion, caval gas embolism was again detected though no signs of bends were seen. This was repeated one week later using the same animal, with the same results. Recompression to 100 feet and stage decompression reduced the rate, but did not eliminate embolic signals; embolic "showers" followed each movement of the animal during stage decompression.

These findings, coupled with the fact that sixteen of nineteen 180 feet/20 minute excursions produced no signs of bends, leave room for little doubt that rather severe caval gas embolism can exist in the

absence of bends signs. Whether caval embolism can exist prior to or in the absence of bends symptoms (in man) remains unknown, however it most probably can. Examination of this possibility will require the use of human subjects and, presumably, catheter-tip transducers with omnidirectional properties. A compromise is conceivable, however, in which caval embolism of experimental (large) animals may correlate with bends incidence in human divers undergoing identical hyperbaric excursions. Presumably human and animal blood have similar surface tension, so that embolism in an animal of similar size and cardiovascular makeup would suggest that the same thing is happening (or about to happen) in his human chambermate.

In Vitro Experiments on Bubble Sizing

In vitro studies continue, attempting to determine whether there exists a useful correlation between gas embolus size and some characteristic of the Doppler signal produced by that bubble. The practical problems have been manifold, and at this time it appears doubtful that the Doppler instrument as it presently exists can give even rough approximations of bubble size. A large number of variables exist which complicate the issue, examples being:

1. Injected artificial emboli (plastic or glass beads or microballoons) have a distribution in size about a mean value; histograms on several graded batches have ranged from left- to right-skewed Gaussians.

2. The beads or balloons tend to clump together; prewashing with detergent minimizes this apparently electrostatic phenomenon.
3. The common acoustic field of the two Doppler transducer crystals is an ill-defined region in the vicinity of the vessel axis; all other things being equal, like size bubbles traversing the vessel will produce different signals depending on their location in the lumen. Related to this is the fact that solid microbeads, hollow microballoons, and probably gas emboli are influenced by gravity effects and tend to roll along vessel walls at low flow rates.
4. Bubbles may pass through the Doppler transducer field at angles to, rather than parallel to, the lumen axis. The component of velocity toward either crystal is therefore variable even at constant flow rates, and as covered in the next paragraph, this affects signal size.
5. The tuned radio frequency receiver in existing Doppler flowmeters does not have a flat frequency response curve; it is not designed to be an "amplitude" device, since flow velocity is related only to the <u>frequency</u> of the audio output. Thus the size of signal produced by a moving embolus varies with flow velocity.
6. Exciter output and TRF receiver gain vary with supply voltage;

it was established that the peak-to-peak amplitude of the final audio output may vary up to 8% with only a 3% change in supply voltage. Thus batteries are unsatisfactory and must be replaced with a well-regulated low voltage power supply.

7. Exciter frequency also changes with supply voltage; proportionate changes in the Doppler shift frequency will also occur. This effect is negligible when using the instrument as a blood flowmeter, however it may confound attempts to relate spectral characteristics of the audio with bubble size.
8. Capacitive coupling exists between the transducer leads to a great extent, so that slightly different lead orientations will affect flowmeter output voltage. Shielded leads or coaxial cables, besides reducing power to the exciter crystal and TRF receiver input, do not eliminate this problem completely.
9. If a telemetric flowmeter is used, variations in the gain of the transmitter and FM receiver will affect signal size. In such systems it is also necessary to flatten the FM receiver response curve by proper adjustment of bass and treble controls.
10. When working with very small (under 50 micra) beads/balloons, the water/glycerin carrier must be filtered and outgassed to eliminate false signals due to microscopic gas bubbles.

Some of these complicating factors can be controlled *in vitro*, however some cannot. *In vivo*, of course, only a few can be controlled

(e.g. items 6, 7, 9), many cannot (e.g. items 3, 4, 5, 8). The most obvious new complication to arise in vivo is the presence of a large blood flow background signal. It has been verified that the ear can discriminate better than moderately sophisticated electronics equipment -- bubbles can be heard which are not visually detectible in the audio signal on an oscilloscope screen.

The first bench system with any measure of success consisted of a closed loop of Silastic tubing containing filtered, outgassed 35% glycerin solution (which has rheologic similarities to whole blood) and a small number of graded microballoons. Near-constant flow was produced by a roller pump built from an old knife sharpener, and flow rate was controlled by a variable transformer providing power to the "pump" motor. Clear signals were obtained and displayed on an oscilloscope. In addition, a half-wave rectifying circuit was used to display the envelope of each microballoon signal on a fast-response strip chart recorder. A large number of observations confirmed that both maximum signal amplitude and signal energy in each "chirp" varied from some maximum value to vanishingly small.

Suspecting that this was largely due to the fact that the particles were passing through the transducer field at different points (item 3 above), a second bench system was devised. The transducer was placed on a length of very thick-walled tubing (12 mm O.D., 2 mm I.D.) and the

glycerin solution allowed to flow through by gravity from a large reservoir. This ensured that all microballoons would be travelling very near and parallel to the axis of flow, and that flow velocities would be quite constant. Again, however, the signals produced by the microballoons were of widely varying sizes.

There is no reason to expect that the scattering properties of glass microballoons and gas emboli are similar at the frequencies employed in these studies, of course. If resonance effects are in play, slight variations in the shell of the microballoons will presumably affect scattering significantly, even if the average shell diameters are equal. Apparatus has been described[9] which produces gas emboli of known and constant size, ejecting them at regular intervals on the axis of a moving fluid stream. We plan to construct a third bench system of this type for future in vitro research.

Obviously there is little sense in constructing elaborate apparatus for the sole purpose of avoiding in vitro problems which cannot be avoided in vivo. While redesigned Doppler instrumentation and more sophisticated signal analysis apparatus may yet prove that Doppler-shifted ultrasound scattered from emboli can give reasonable estimates of their size, the time and expense involved in seeking this goal overwhelmingly exceeds the scope of our current program. We are merely attempting to demonstrate whether the Doppler flowmeter as it now exists, plus relatively ordinary electronics backup, has any promise

as an *in vivo* bubble sizer. If bubbles of identical size moving at constant velocity down the axis of a tube produce a broad spectrum of varying output signals, all other factors being controlled to whatever extent possible, we will have answered that question.

FUTURE PLANS

We have fairly well bracketed the no-decompression limits (based on signs) for miniature swine at 140 and 180 feet. Future pig-dives are directed toward a statistically sound definition of these limits and those at 60 and 100 feet. Preliminary observations indicate that 100 feet/90 minutes produces a very high incidence of bends in miniature swine.

Continued in vitro work on Doppler bubble sizing is planned, utilizing axial flow of calibrated gas bubbles as signal sources. If time and funds permit, we shall investigate means for increasing the ratio of embolus signal size to blood flow background and perform additional in vivo Doppler studies on pig-dives which do not produce signs of bends. We are particularly interested in seeing whether the tendency of blood to nucleate depends on prior diving experience.

Surgeon Commander Rawlins' suggested profile proved to be extremely worthwhile, indicating that miniature swine are perhaps similar to R. N. divers in their susceptibility to bends. We request further suggestions of this type: no-decompression (or otherwise) profiles which produce a known incidence of bends in human subjects. To whatever extent possible we will try them on our minipigs.

Future plans tentatively include the development of no-decompression limits for miniswine based not on signs, but on the appearance of gas emboli in the caudal vena cava or pulmonary artery. The bottom times

allowable by this method will be less than those established on the basis of clinical signs, and it is hoped such limits correlate more closely with symptomatic limits established for man. Because of funding limitations, this work will necessarily be deferred until the third fiscal year.

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